Elatinaceae are Sister to Malpighiaceae; Peridiscaceae Belong to Saxifragales

American Journal of Botany 91(2): 262–273. 2004.

ELATINACEAE ARE SISTER TO MALPIGHIACEAE; PERIDISCACEAE BELONG TO SAXIFRAGALES[1]

CHARLES C. DAVIS[2,4] AND MARK W. CHASE[3]

[2]Department of Ecology and Evolutionary Biology, University of Michigan Herbarium, 3600 Varsity Drive, Ann Arbor, Michigan 48108-2287 USA; and [3]Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS UK

Phylogenetic data from plastid (*ndhF* and *rbcL*) and nuclear (*PHYC*) genes indicate that, within the order Malpighiales, Elatinaceae are strongly supported as sister to Malpighiaceae. There are several putative morphological synapomorphies for this clade; most notably, they both have a base chromosome number of $X = 6$ (or some multiple of three or six), opposite or whorled leaves with stipules, unicellular hairs (also uniseriate in some Elatinaceae), multicellular glands on the leaves, and resin (Elatinacae) or latex (Malpighiaceae). Further study is needed to determine if these features are synapomorphic within the order. Malpighiaceae have previously been inferred as sister to Peridiscaceae based on *rbcL* sequence data, but the *rbcL* sequence of *Whittonia* is a chimera of two sequences, neither of which appears to be *Whittonia*. Our data from plastid (*atpB, rbcL*) and nuclear (18S rDNA) genes instead place Peridiscaeace as a member of the Saxifragales.

Key words: *Bergia*; *Elatine*; Malpighiales; *ndhF*; *Peridiscus*; *PHYC*; Saxifragales; *Whittonia*.

Tremendous progress has been made in clarifying the higher level placement of most angiosperm families (APG, 1998, 2003; Soltis et al., 2003), yet resolution within many eudicot orders remains problematic. Several notable studies have built on existing phylogenetic data, and the incorporation of additional genes and (or) increased taxonomic sampling have successfully identified several family pairs involving taxa of uncertain affinities (Vochysiaceae with Myrtaceae in Myrtales, Conti et al., 1996; Euphroniaceae and Trigoniaceae with Chrysobalanaceae in Malpighiales, Litt and Chase, 1999; Aphloiaceae with Ixerbaceae in a so-far unnamed clade, Soltis et al., 2000; Hydrostachyaceae with Hydrangeaceae in Cornales, Albach et al., 2001; Tepuianthaceae in Thymelaeaceae [in Malvales], Wurdack and Horn, 2001; and Hydnoraceae with Aristolochiaceae in Aristolochiales, Nickrent et al., 2002). Conversely, efforts to identify the closest relative of Malpighiaceae have been less successful despite extensive sampling (Savolainen et al., 2000a, b; Soltis et al., 2000; Chase et al., 2002; Wurdack, 2002). Malpighiaceae are a clade of ~1300 species of trees, shrubs, and vines found in the tropics and subtropics of both hemispheres. Approximately 85% of the diversity of the family is found in the New World. The monophyly of Malpighiaceae is well supported by morphological (Anderson, 1979, 1990) and molecular evidence (Savolainen et al., 2000a, b; Soltis et al., 2000; Chase et al., 2002; Wurdack, 2002), but the family is morphologically isolated from other rosids (Anderson, 1990).

Malpighiaceae are characterized by many autapomorphies, making it difficult to identify their closest sister group. Especially distinctive are their unusual floral morphology (Anderson, 1979, 1990; Vogel, 1990; Fig. 1a, b) and the presence of unicellular T-shaped hairs (see Fig. 1c). They share a suite of floral characteristics including clawed (or paddle-shaped) petals, one of which is oriented out of the plane of the others (the ''flag'' petal), and sepals with paired, abaxial glands that produce oils (in New World taxa) or nectar (in some Old World taxa). In its most characteristic New World form (e.g., Fig. 1a, b), this floral morphology is associated with pollination by oil-collecting anthophorine bees (Vogel, 1974, 1990; Anderson, 1979, 1990; Neff and Simpson, 1981; Taylor and Crepet, 1987). Neotropical Malpighiaceae appear to have coevolved with these insect pollinators, and this may partially account for the greater diversity of New World species relative to Old World species. Until the sister-group relationships of the family are clarified we cannot begin to evaluate the hypothesis that a shift in species diversification was associated with these novel floral structures associated with the family (e.g., Guyer and Slowinski, 1993; Sanderson and Donoghue, 1994; Mooers and Heard, 1997, 2002; Sims and McConway, 2003).

The taxonomic history since the first recognition of the family over 200 years ago (de Jussieu, 1789) up through the most recent phylogenetic investigations (e.g., Chase et al., 2002; Wurdack, 2002) reflect this impasse. Whereas previous phylogenetic studies of plastid (*rbcL, atpB*) and nuclear ribosomal 18S DNA (rDNA; Chase et al., 1993, 2002; Savolainen et al., 2000a, b; Soltis et al., 2000) have done a great deal to clarify membership of Malpighiales, none of them has resolved the sister to Malpighiaceae with high internal support (greater than 80% bootstrap or jackknife). Savolainen et al. (2000b) found that a small South American family, Peridiscaceae (two genera, *Peridiscus* and *Whittonia*), are sister to Malpighiaceae, a finding that although not initially well supported (<50% bootstrap) has gained increasing support in more recent studies with improved taxonomic sampling (Chase et al., 2002; Wurdack, 2002). It is now clear that the *rbcL* sequence used in

[1] Manuscript received 29 May 2003; revision accepted 29 August 2003.

K. Wurdack and A. Amorim kindly provided genomic DNAs for this study. K. Wurdack also provided helpful suggestions to improve our sampling of ''Euphorbiaceae s.l.'' and shared part of his thesis on Euphorbiaceae phylogeny. W. Anderson, C. Davis, W. Judd, S. Mathews, and P. Stevens reviewed and commented on an early version of this manuscript. Two Harvard undergraduates, H. Jonnson and M. Ovadia, helped collect data for this study. P. Tucker and D. Mindell provided lab facilities in the Ruthven Museums to complete this work. Reference material was loaned by the following herbaria: GH, MICH, MO, and NY. Illustrations were prepared by K. Douthit, and Fig. 1 was reproduced with the permission of W. Anderson and the University of Michigan Herbarium. Funding was provided to C. C. D. by a Deland Award from the Arnold Arboretum, NSF Doctoral Dissertation Improvement grant DEB-0073299, and a Rackham Faculty grant from the University of Michigan. The Michigan Society of Fellows helped C. C. D. complete this study. This paper is dedicated to the memory of Warren ''Herb'' Wagner.

[4] E-mail: chdavis@umich.edu.

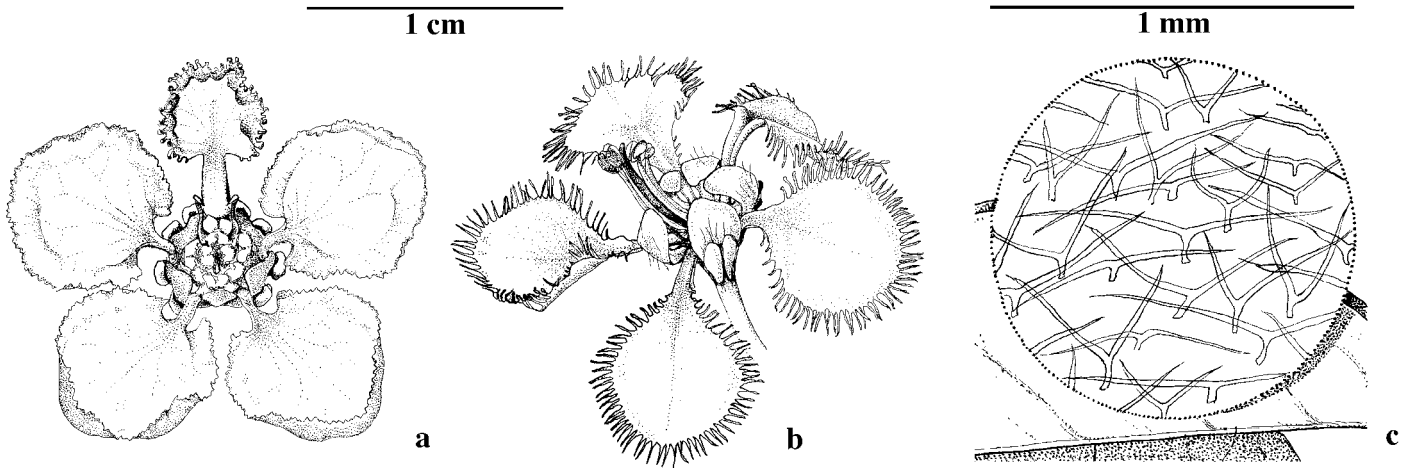

Fig. 1. Details of neotropical malpigh flowers (a–b) and vesture (c). (a) Single upright ''flag'' petal and the characteristic paddle-shaped petal morphology found in most Malpighiaceae (from *Hiraea bullata* [W. R. Anderson; (W. R. Anderson, University of Michigan Herbarium, unpublished, copyright W. R. Anderson]), (b) paired abaxial sepal glands, which produce oils (from *Janusia schwannioides* W. R. Anderson; Anderson [1982, fig. 14]), (c) vesture detail showing the characteristic medifixed, or T-shaped hairs, which are prominent on both vegetative and reproductive tissues in members of the family (from *Malpighia rzedowskii* W. R. Anderson; Anderson [1987, fig. 14]).

these studies is a chimera; the first 628 base pairs (bp) are derived from a contaminating DNA of a member of Malpighiaceae (perhaps a *Byrsonima*), and the last 742 bp may be from a member of the clusioids (sensu Wurdack, 2002; M. W. Chase, personal observation; results not shown here).

The aim of this paper is to document the sister group of Malpighiaceae, which will help set the stage for future investigations examining diversification patterns within Malpighiaceae relating to floral characters that have long been thought to explain the neotropical diversity of the family. We have collected new data from the nuclear, protein-coding, phytochrome gene, *PHYC,* and the plastid genes *ndhF* and *rbcL* from species representing all families within Malpighiales (APG, 2003). These genes have been used for examining infrafamilial relationships of Malpighiaceae (Cameron et al., 2001; Davis et al., 2001, 2002a, b; Davis, 2002) and were thought to be useful for examining phylogenetic relationships among families in Malpighiales. In addition, we obtained recently collected material of Peridiscaceae and included it in a combined analysis using the Soltis et al. (2000) three-gene matrix to infer their phylogenetic placement.

MATERIALS AND METHODS

Taxon sampling—The familial- and ordinal-level circumscriptions for this study follows the APG (2003) system. The taxa and voucher information (including GenBank accession numbers) for these analyses may be found in Appendix 1 (see Supplemental Data accompanying the online version of this article).

We sampled *ndhF, rbcL,* and *PHYC* from species representing all of the families of Malpighiales (APG, 2003; Appendix 1). The sampling strategy was guided by several recent phylogenetic analyses based on *rbcL* (Savolainen et al., 2000b; Chase et al., 2002); *rbcL* plus *atpB* (Savolainen et al., 2000a); and *rbcL, atpB,* and 18S rDNA (Soltis et al., 2000; Wurdack, 2002). Our goal was to maintain taxonomic compatibility with these earlier studies, especially with Savolainen et al. (2000b) and Wurdack (2002), so that we can combine these data sets in the future.

We assembled *ndhF* from 65 species representing outgroups and all major families within Malpighiales (APG, 2003). Five of these sequences were published by Davis et al. (2001, 2002b). We obtained *rbcL* from 70 species from the same subset of taxa, most of which were published by Chase et al. (2002). Lastly, we sequenced *PHYC* from 51 individuals representing the same families described, five of which were published by Davis et al. (2002b).

We were unable to amplify *PHYC* or *ndhF* from the DNA of *Whittonia guianensis* Benth. used by previous authors, perhaps because only highly degraded DNA was extracted from this herbarium material. Preliminary phylogenetic analyses using fresh samples of Peridiscaceae (*Peridiscus lucidus* Benth.) in which this taxon was specified as ingroup placed it well outside of the core Malpighiales (APG, 2003). As an additional assessment of the placement of Peridiscaceae we sampled the same material for *rbcL* (see Appendix 1 in the Supplemental Data accompanying the online version of this article), *atpB* (GBANK-AY372816), and 18S rDNA (GBANK-AY372815) and inferred its phylogenetic placement using the 567-taxon data set of Soltis et al. (2000). Analysis of this data set indicated that Peridiscaceae is a member of Saxifragales. In our final analyses of the *ndhF, rbcL,* and *PHYC* data, we used *Peridiscus* as an outgroup to Malpighiales, not as a member of the ingroup.

Broader, angiosperm-wide analyses (Soltis et al., 2000, 2003) of multiple genes have indicated that Celastrales are closely related to Malpighiales. We sampled five species of Celastraceae to use as outgroups (see Appendix 1 in Supplemental Data accompanying the online version of this article). Also, we included the non-rosid Dilleniaceae as an additional outgroup (Soltis et al., 2000, 2003; Savolainen et al., 2000a).

Molecular and phylogenetic methods—Protocols for extracting DNA, amplification of *ndhF* and *PHYC,* cloning (for *PHYC*), and automated sequencing generally followed those reported by Davis et al. (2002a and references cited within; but see also Davis et al. 2001, 2002b). Amplification and sequencing primers for *rbcL, atpB,* and 18S rDNA followed Chase et al. (2002, and references within), Hoot et al. (1995), and Soltis and Soltis (1997), respectively. Nucleotide and amino acid sequences were aligned by eye, and the ends were trimmed from each data set to maintain complementary data between taxa. The *ndhF, rbcL,* and *PHYC* data sets were analyzed independently, as a single plastid (*ndhF* plus *rbcL*; plastid DNA) and nuclear data set (*PHYC*) and in combination using parsimony as implemented in PAUP* version 4.0b10 (Swofford, 2000), with 100 random taxon addition replicates, tree-bisection-reconnection (TBR) branch swapping, and MulTrees in effect. Gap positions were treated as missing data; all characters were weighted equally, and character states were unordered (Fitch parsimony; Fitch, 1971). Bootstrap support (BS; Felsenstein, 1985) for each clade was estimated from 100 heuristic search replicates as described above, but with simple taxon addition in effect.

Due to the excessive number of trees and computational time searching on the three-gene, 567-taxon data set of Soltis et al. (2000), we held only 10

trees in each replicate. Similarly, bootstrap percentages were calculated for this data set using the ''fast'' bootstrap option. After we approximated the placement of *Peridiscus*, we reduced the data set to a subset of taxa representing its closest relatives (Saxifragales) and outgroups (Caryophyllales) based on the analyses by Soltis and Soltis (1997), Fishbein et al. (2001), and Soltis et al. (2003). Searches and bootstrap percentages for this smaller data set were conducted in the same manner as described previously for the independent and combined data sets.

Searches on the plastid DNA data set were conducted using only taxa sampled for both genes. Searches using the combined ''expanded'' plastid DNA and *PHYC* data included all taxa for which at least one gene was available. This ensured that all of the families within Malpighiales were sampled. Some *PHYC* sequences were difficult to obtain for some members of the order because it is likely that *PHYC* is absent from some families. Howe et al. (1998) presented evidence that *Populus trichocarpa* Torr. and Gray does not have representatives of the *PHYC* gene subfamily; *PHYC* type genes were not detected by PCR, screening of cDNA libraries, or Southern analyses. It is not clear where this loss (or losses) may have occurred within Salicaceae nor how widespread it may be, but based on results from PCR at least some families closely related to Salicaceae have also apparently lost the gene (C. C. Davis, unpublished data); they include Achariaceae, Turneraceae, Passifloraceae, and Malesherbiaceae, which form a clade with Salicaceae (Chase et al., 2002; Wurdack, 2002; this study). However, we were able to obtain *PHYC* from at least one tropical member of Salicaceae, *Dovyalis*. This result was unexpected given the apparent absence of *PHYC* from *Populus trichocarpa* and families related to Salicaceae (S. Mathews, Arnold Arboretum, personal communication). It is possible that *PHYC* is present, but unamplifiable with our primers/protocols, in these related families, and the loss is restricted to a clade within Salicaceae. Alternatively, there may have been multiple losses of the gene within families closely related to Salicaceae. Outside of Salicaceae and their closest relatives mentioned, this does not appear to be a major problem, i.e., *PHYC* was easily obtained from other related families. None of the groups in which *PHYC* has putatively been lost are closely related to Malpighiaceae, which is confirmed by available plastid DNA sequences presented here as well as by broader analyses by Wurdack (2002).

RESULTS

Sequences/matrices—The aligned *ndhF*, *rbcL*, and *PHYC* sequences are 811, 1428, and 1189 bp long, respectively. The alignment of *ndhF* included several indel regions of varying lengths, whereas *rbcL* included none. The combined plastid DNA data set was 2103 bp long (with ends trimmed) and included 63 taxa. *PHYC* included only three indel regions. The alignment of these sequences was aided by the use of amino acid translations and is available in Appendix 2 (see Supplemental Data accompanying the online version of this article).

The bootstrap consensus tree generated from the independent data sets revealed no ''hard incongruence'' (Whitten et al., 2000; Reeves et al., 2001); that is, we found no strongly supported (≥90%) incongruent clades between the independent analyses of the plastid DNA (Fig. 2) and *PHYC* (Fig. 3) data sets. We subsequently analyzed these data in combination. The combined 3428 bp long (3258 bp after trimming ends) ''expanded'' data matrix included 72 taxa (65 ingroup plus seven outgroups) and 1973 variable (1478 potentially parsimony informative) characters.

Phylogenetic analyses—Analyses of the independent and combined data sets yielded similar results. Both independent analyses support the grouping of Elatinaceae plus Malpighiaceae with ≥90% BS (Figs. 2, 3), and the combined ''expanded'' analysis supports that same grouping with similar support (94% BS; Fig. 4). Resolution along the spine of the Malpighiales tree was poor in these analyses (Figs. 2–4). The sister to the Malpighiaceae-Elatinaceae clade is not strongly supported in any analysis, but in the *PHYC* trees Picrodendraceae are sister to this clade (Fig. 3; 60% BS).

In the three-gene analysis of the reduced Soltis et al. (2000) data set, Peridiscaceae were placed with high BS support (98%) as a member of the Saxifragales (Fig. 5). Within the order, there were few well-resolved nodes, but these data indicate that Peridiscaceae are sister to Paeoniaceae (BS < 50%; Fig. 5). The alignment of these sequences are available in Appendix 3 (see Supplemental Data accompanying the online version of this article).

DISCUSSION

Peridiscaceae do not belong in Malpighiales—The small, poorly known family Peridiscaceae contain two monotypic genera; *Peridiscus lucidus* and *Whittonia guianensis* are both from northern South America. The former is endemic to Amazonian Brazil (Bentham and Hooker, 1862) and the latter to Guyana (Sandwith, 1962). There appears to be little doubt that Peridiscaceae are monophyletic based on the many vegetative, reproductive, and anatomical features shared between these two genera (Sandwith, 1962; Metcalfe, 1962). The finding of a Perdiscaceae/Malpighiaceae clade was based on the *rbcL* sequence of *Whittonia* in Savolainen et al. (2000b; GenBank accession number AJ403018), but this sequence is a chimera, and neither half appears to be from *Whittonia*. Prior to this study, the placement of Peridiscaceae was mostly inconclusive, but most modern systematists (Sandwith, 1962; Cronquist, 1981; Thorne, 1992b; Takhtajan, 1997) speculated that their affinities were with Flacourtiaceae. Most of the former flacourt genera belong to Malpighiales (APG, 1998, 2003), but that family has recently been shown to be polyphyletic (Chase et al., 2002).

Our study indicates that Malpighiaceae are not closely related to Peridiscaceae. Instead, Peridiscaceae are placed well outside of Malpighiales and should not be included in future circumscriptions of the order; they are members of Saxifragales (Fig. 5). Placement of Peridiscaceae in Saxifragales is not only supported by high bootstrap support (98%; Fig. 5), but also by the presence of a unique indel in the 18S rDNA gene found only among members of Saxifragales within eudicots, which is synapomorphic for the order (indel B, table 3 in Soltis and Soltis, 1997). Additional data are needed to resolve the placement of Peridiscaceae within Saxifragales, but investigations (Fishbein et al., 2001) on this group have indicated that resolving the higher level relationships within the order may be difficult due to their apparently rapid (and ancient) radiation.

Morphological synapomorphies for Saxifragales have been difficult to address conclusively, which makes it problematic to identify clear morphological characters supporting the placement of Peridiscaceae within the order. Performing such an analysis is outside the scope of this paper, but we did make some comparisons of characters (Cronquist, 1981; Table 1) found in Peridiscaceae with those of two sets of families: (1) those to which Peridiscaceae have been previously suggested to have a close relationship, namely Flacourtiaceae (sensu Cronquist, 1981), and (2) primarily woody families with at least some tropical affinities, such as Daphniphyllaceae and Hamamelidaceae. There are a number of characters among these two woody saxifragalean families that are similar to those in Peridiscaceae, whereas those shared with Flacourti-

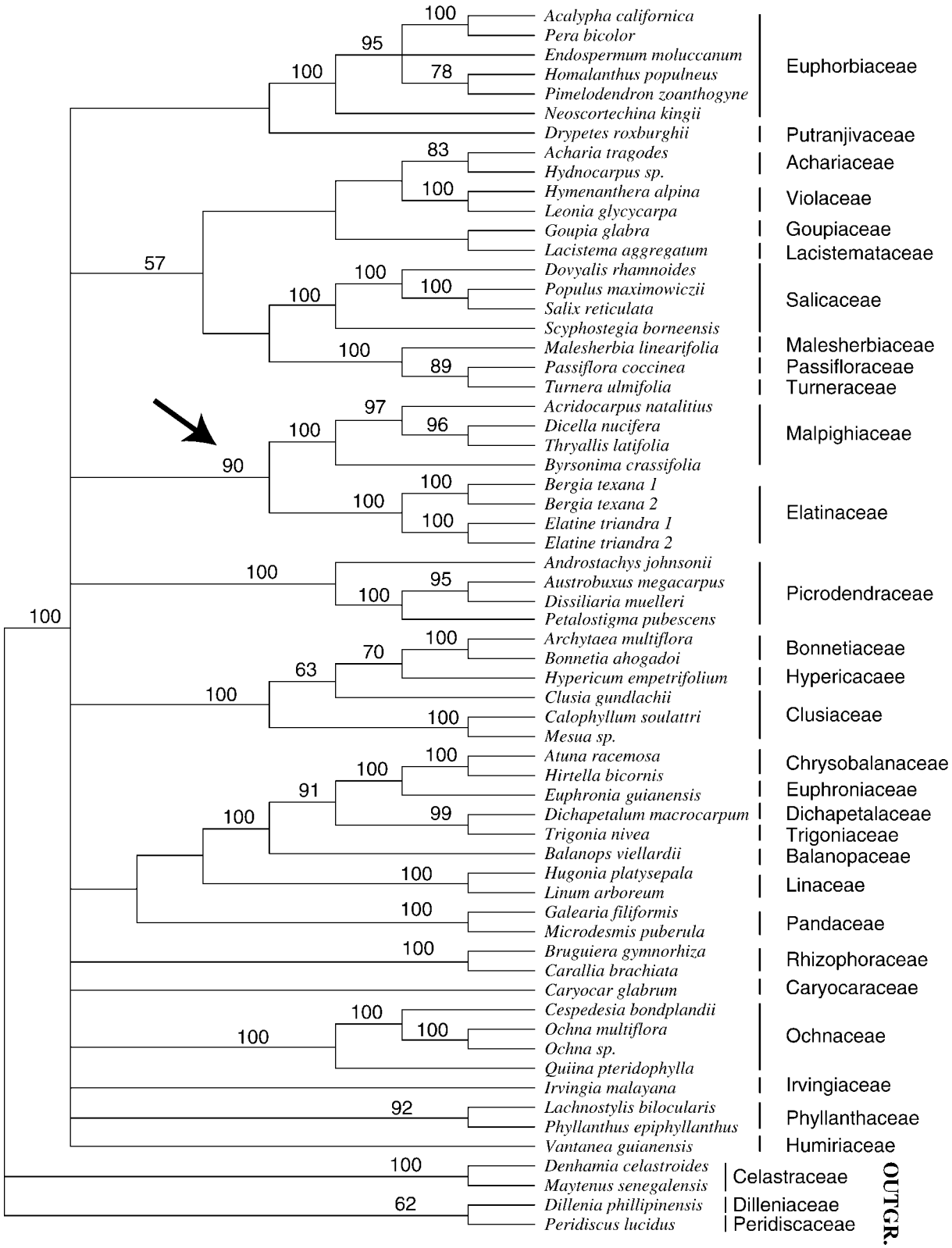

Fig. 2. Strict consensus tree of 26 most parsimonious trees resulting from the independent analysis of the pooled plastid DNA data (*ndhF* and *rbcL*). Tree length = 4680; CI = 0.39; RI = 0.50. Numbers above branches are bootstrap percentages >50%. The arrow indicates the Malpighiaceae plus Elatinaceae clade, which receives 90% bootstrap support.

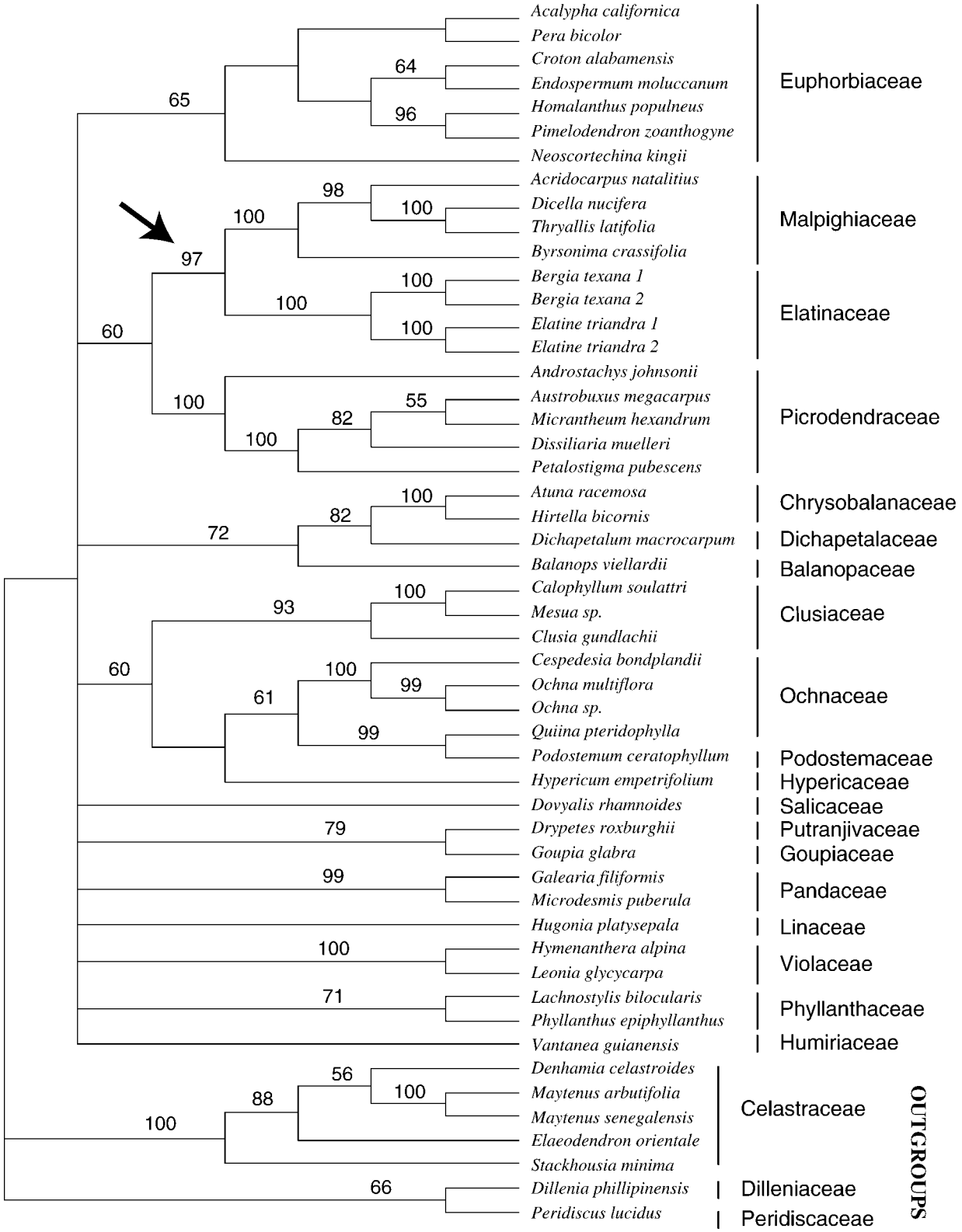

Fig. 3. Strict consensus tree of the 16 most parsimonious trees resulting from the independent analysis of the nuclear *PHYC* data. Tree length = 4393; CI = 0.35; RI = 0.45. Numbers above branches are bootstrap percentages >50%. The arrow indicates the Malpighiaceae plus Elatinaceae clade, which receives 97% bootstrap support.

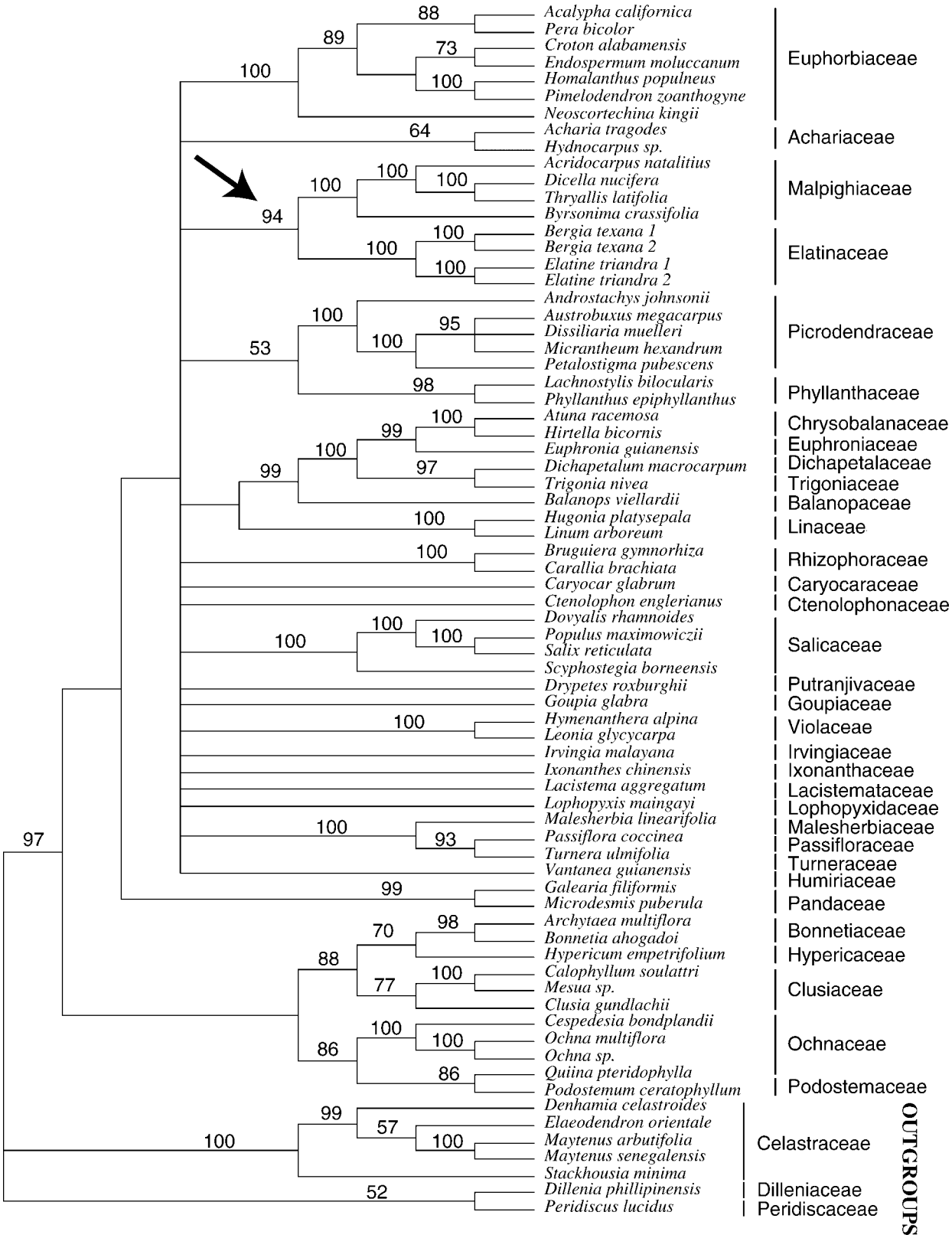

Fig. 4. Strict consensus tree of the 65 most parsimonious trees resulting from the analysis of the combined ''expanded'' data set. All of the families of Malpighiales are represented in this tree. Tree length = 9528; CI = 0.37; RI = 0.47. Numbers above branches are bootstrap percentages >50%. The arrow indicates the Malpighiaceae plus Elatinaceae clade, which receives 94% bootstrap support.

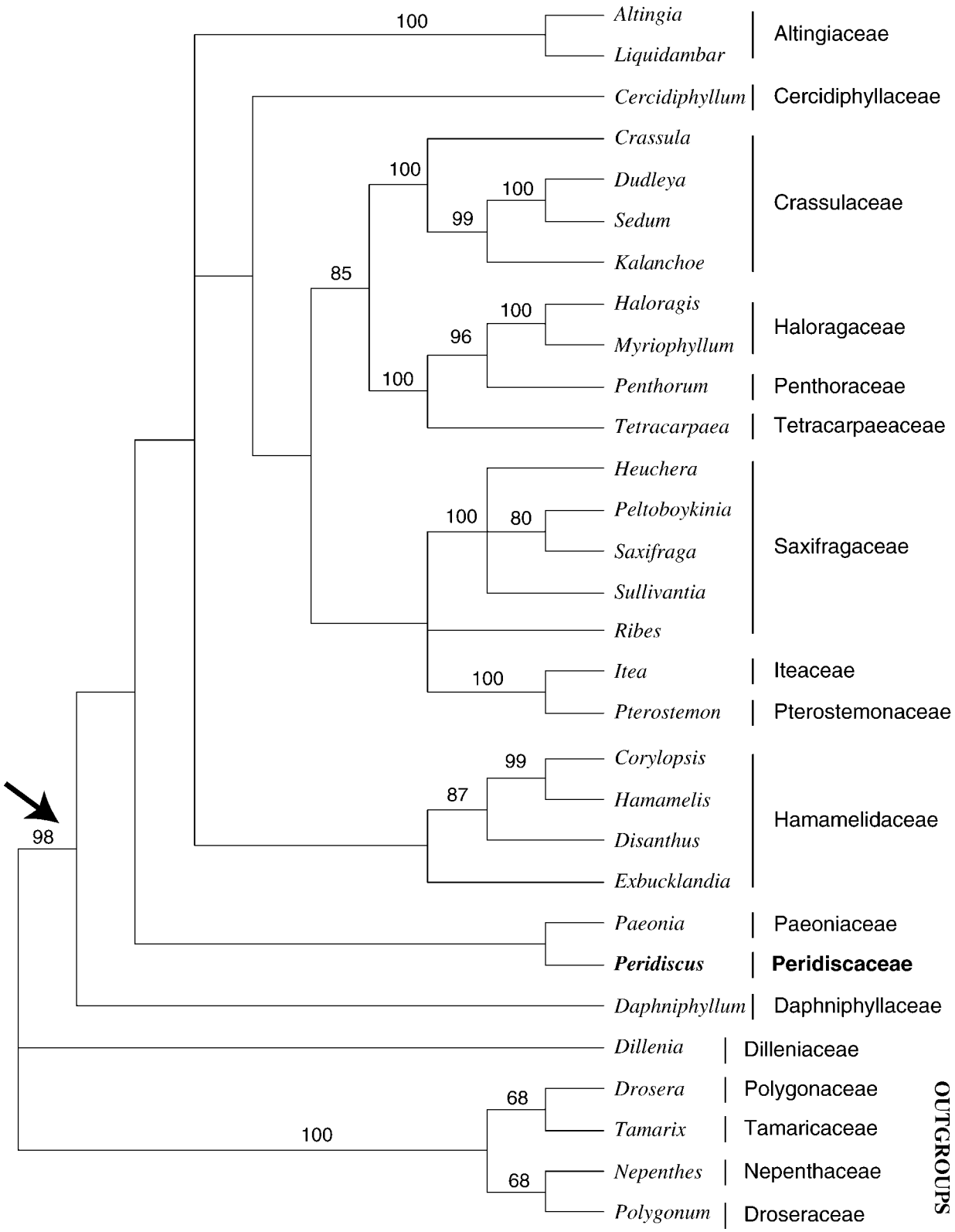

Fig. 5. Strict consensus tree of the eight most parsimonious trees resulting from the combined analysis of *atpB*, *rbcL*, and 18S rDNA data on the Saxifragales data set. Tree length = 2251; CI = 0.59; RI = 0.55. Numbers above branches are bootstrap percentages >50%. The arrow indicates the Saxifragales clade, which receives 98% bootstrap support. Peridiscaceae is shown in bold.

TABLE 1. Comparison of Peridiscaceae with some representative families of Saxifragales as well as Flacourtiaceae, with which it has often been allied (characters taken from Cronquist, 1981).

Character	Peridiscaceae	Daphniphyllaceae	Hamamelidaceae	Flacourtiaceae
Calcium oxalate crystals in parenchymatous tissues	present in some	present in some	present in some	if present, in idioblasts
Primary veins	palmate	pinnate	palmate	pinnate
Stomates	anomocytic	paracytic	various	paracytic or anisocytic
Stipules	present	absent	present	present
Scalariform perforation plates	present	present	mostly present	present in some genera
Tracheary elements	imperforate with bordered pits	imperforate with bordered pits	imperforate with bordered pits	imperforate with bordered pits
Wood parenchyma	diffuse	diffuse	apotracheal, diffuse	absent
Inflorescence type	axillary fascicles/racemes	axillary racemes	spikes (seldom racemose or paniculate)	various
Petals present/absent	absent	absent	usually present	usually present (not in Saliceae)
Floral subtending bract	present	present	absent	absent
Sepals	4–7, imbricate	2–6 (rarely absent), imbricate	4–5 (–10), imbricate	3–8 (–15), imbricate or valvate
Stamens	numerous, anther dehiscence by slits	5–6, anther dehiscence by slits	4–5 (–10), anther dehiscence by slits/valves	3–8 (–15), anther dehiscence by slits (rarely porose)
Pollen	tricolporate	tricolporate	tricolpate (to sometimes multiporate)	tricolporate, tricolpate, or triporate
Carpel number	3–4	2(–4)	2(3)	2–10
Ovary position	half inferior	superior	half to fully inferior	superior (rarely inferior)
Style length	short	short	long	long
Ovules	pendulous from top of the ovary	pendulous from top of the ovary	pendulous from top of the ovary	parietal
Fruit	one-seeded drupe	one-seeded drupe	capsular	berry, rarely a capsule or drupe
Embryo	small, ?	small, straight	large, straight	?, straight (mostly)

aceae in the broad sense used by most previous authors (e.g., Cronquist, 1981) are as often as not also shared by families in Saxifragales (Table 1). It appears that Peridiscaceae are not out of place in Saxifragales, and we await the results of further phylogenetic and morphological studies focused on characters found in the families of this order, including Peridiscaceae.

Elatinaceae are sister to Malpighiaceae—Our analyses clearly place Malpighiaceae as sister to Elatinaceae (Figs. 2–4). Elatinaceae are a bigeneric family of cosmopolitan aquatic herbs or terrestrial shrubs; *Elatine* L. and *Bergia* L. have between them 35–50 species (Tucker, 1986; Leach, 1989). *Elatine* contains 12–25 species and is most diverse in the temperate zone of both hemispheres (Tucker, 1986): 12 species are found in Eurasia (three of which are also found in northern Africa), 10 in North America, 5–7 in South America (mostly in temperate to montane zones), two species in India and Malesia, two in southern Africa, and one in Australasia. *Bergia* contains ~25 species and is most diverse in the Old World tropics, principally Africa and Australia (Tucker, 1986; Leach, 1989): 10–20 species occur in eastern and southern Africa, 10 in Australia, five species in southern Asia, two in Malesia, and three species in the New World tropics (with one species, *Bergia texana*, extending into temperate North America).

The previous taxonomic classification of Elatinaceae reflects their uncertain phylogenetic position. Adanson (1764) placed *Elatine* in Caryophyllaceae due to their possession of opposite leaves, small flowers, and tiny seeds. Several authors (de Candolle, 1824; Bentham and Hooker, 1862; Bessey, 1915; Hutchinson, 1926, 1959) subsequently followed Adanson and placed the family in (or near) Caryophyllaceae. Others (Niedenzu, 1925; von Wettstein, 1935) suggested that Elatinaceae were instead closely related to Frankeniaceae and Tamaricaceace, a placement that has been justified on the basis of anatomical, palynological, and embryological evidence (Walia and Kapil, 1965; Melikian and Dildarian, 1977). This latter relationship was always doubtful, however, given the widespread distribution of characters supporting this hypothesis (Tucker, 1986). Cambessèdes (1829) and Gray (1849) departed from all previous treatments and suggested that the family was instead closely allied with Clusiaceae sensu lato (s.l.; including Hypericaceae). Most modern treatments of Elatinaceae (Thorne, 1976, 1983, 1992a, b; Taktahjan, 1980, 1997; Dahlgren, 1980; Cronquist, 1981) have followed Cambessèdes and Gray by similarly placing the family near Clusiaceae s.l.

Recent phylogenetic studies (Savolainen et al., 2000b; Chase et al., 2002) have corroborated evidence by Cambessèdes (1829) and subsequent authors that Clusiaceae and Elatinaceae are more closely related than either is to Caryophyllaceae, Frankeniaceae, or Tamaricaceace; both Clusiaceae and Elatinaceae are members of Malpighiales (APG, 2003). However, whereas several studies (Savolainen et al., 2000b; Chase et al., 2002; Wurdack, 2002) indicated that Elatinaceae are monophyletic, the placement of the family within Malpighiales has remained unclear. Savolainen et al. (2000b) initially inferred the clade ((Malpighiaceae, Peridiscaceae) (Phyllanthaceae, Elatinaceae)) based on *rbcL*. Although there was BS support <50% for (and within) that clade, except for those clades uniting the three genera of Malpighiaceae (98% BS) and the two genera of Elatinaceae (54% BS), there was a weakly supported association of Malpighiaceae with Elatinaceae. In the recent analysis by Chase et al. (2002), again based on *rbcL*, Elatinaceae were placed in some of their trees as sister to a clade containing the clusioids (i.e., Podostemaceae, Hyperi-

caceae, Bonnetiaceae, and Clusiaceae), but there was BS <50% for this clade, and the strict consensus of their trees left the placement of Elatinaceae ambiguous. In Wurdack's (2002) broad, three-gene analysis of Malpighiales, which included the widest infraordinal sampling to date, Elatinaceae were paired with Malpighiaceae, but with only 51% BS support. Our study is the first to convincingly place Elatinaceae as sister to Malpighiaceae (Figs. 2–4). In all independent and combined analyses in our study, this clade is inferred with ≥90% BS support (Figs. 2–4).

Morphological evidence for the placement of Elatinaceae with Malpighiaceae—Part of the problem with estimating the relationships of Elatinaceae with any other taxon is that species of *Elatine* are almost entirely aquatic and herbaceous. Aquatic angiosperms represent a diverse assemblage of species, which have arisen from terrestrial ancestors as many as 100 times (Cook, 1999). The highly convergent and reduced nature of most aquatic plants has often made it difficult to interpret their morphology in a phylogenetic context (Philbrick and Les, 1996). Gray (1849, p. 15) commented specifically on this phenomenon in *Elatine* by suggesting that they "... are bland plants, destitute of any marked sensible qualities, as far as is known ..." The mostly temperate distribution exhibited by the aquatic species of *Elatine* has almost certainly biased perceptions of the family and may reflect a broader misconception that most members of Elatinaceae are temperate aquatic herbs. The center of diversity of Elatinaceae is instead found among the paleotropical species of *Bergia* (Tucker, 1986; Leach, 1989), which are mostly woody (many are recorded as shrubs) and are often found in drier or even arid, upland environments. They also tend to offer more obvious vegetative and anatomical characters for evaluation than the highly reduced morphology exhibited by many species of *Elatine*.

There are a number of morphological features shared by Malpighiaceae and Elatinaceae that may be synapomorphic for this clade. The Malpighiaceae characters summarized in Table 2 represent features that are found principally among members of the New World byrsonimoid clade (sensu Davis et al., 2001), for which Anderson (1978, 1990) identified as putatively ancestral for Malpighiaceae. There are a number of parallels between some malpighs and many species of Elatinaceae. Most notably, they both have a base chromosome number of $X = 6$ (or some multiple of three or six, e.g., nine in *Elatine* or 12 to rarely 24 in some byrsonimoids), opposite or whorled leaves with conspicuous stipules borne at or between the petiole bases, unicellular hairs (apparently uniseriate in some Elatinaceae), multicellular glands on the leaves, and resin (Elatinacae) or latex (Malpighiaceae). However, given that the sister group to the Elatinaceae-Malpighiaceae clade is uncertain (Figs. 2–4), these features may be revealed to be symplesiomorphic in studies with increased phylogenetic resolution, or as the phylogenetic relationships of either ingroup becomes better resolved, especially for Elatinaceae, some of the features may later be revealed to be independently evolved in these two taxa.

The presence of stipules in Elatinaceae is suggestive of a close association with Malpighiaceae (Fig. 6a). Despite similar characteristics of wood (Cronquist, 1981) and seed (Corner, 1976) in Elatinaceae and Clusiaceae, the presence of conspicuous stipules in Elatinacae have always made them a bad fit with Clusiaceae (Cronquist, 1981), which are entirely estipulate. In contrast, stipules occur in most species of Malpighiaceae and are morphologically similar to those found in some Elatinaceae. In addition, it has recently been found (Davis et al., 2002b) that representatives of the clade that is sister or paraphyletic to the crown byrsonimoid clade (i.e., representatives of tribe Galphimieae [sensu Anderson, 1978]), possess laticifers (Vega et al., 2002). This discovery led these authors to speculate that Malpighiaceae were most closely related to Euphorbiaceae sensu stricto, a finding that is not supported by this study. Although laticifers have not been reported in Elatinaceae, species of Elatinaceae do posses a brownish resin (Cronquist, 1981; Carlquist, 1984), which may be anatomi-

TABLE 2. Comparison of byrsonimoid Malpighiaceae (Anderson, 1978, 1990, 1993; Vega et al., 2002) and Elatinaceae (Cronquist, 1981; Tucker, 1986).

Character	Elatinaceae	Malpighiaceae (byrsonimoids)
Habit	shrubs or herbs	trees, shrubs, perennial herbs
Base chromosome number	$X = 6, 9$	$X = 6, 12$, rarely 24
Hairs	unicellular or uniseriate, basifixed and sometimes gland-tipped	unicellular, medifixed
Latex	resinous ± throughout	laticifers in Galphimieae
Leaves	opposite or whorled, simple, entire, or toothed	opposite, simple, entire (rarely toothed)
Extrafloral glands	multicellular glands often along leaf margins	multicellular glands variously placed on leaves and stems
Stipules	present, interpetiolar, distinct	present, mostly intra-(epi-)petiolar, distinct or connate
Infloresence	single to several flowered cymules	single to several flowered cymules, often borne in compound racemes
Flowers	bisexual	bisexual
Sepals	2–5 (–6) distinct to half connate	five, distinct or basally connate
Petals	as many as sepals, distinct, imbricate	five, distinct, imbricate
Stamens	as many as sepals or twice as many	10
Pollen	tricolporate	tricolporate
Ovary position	superior	superior
Carpel number	2–5, united	three, distinct to united
Locule number	as many as carpels	as many as carpels
Placentation	axile	axile
Fruit	septicidal capsule	septicidal capsule among some Galphimieae
Seeds	without endosperm	without endosperm

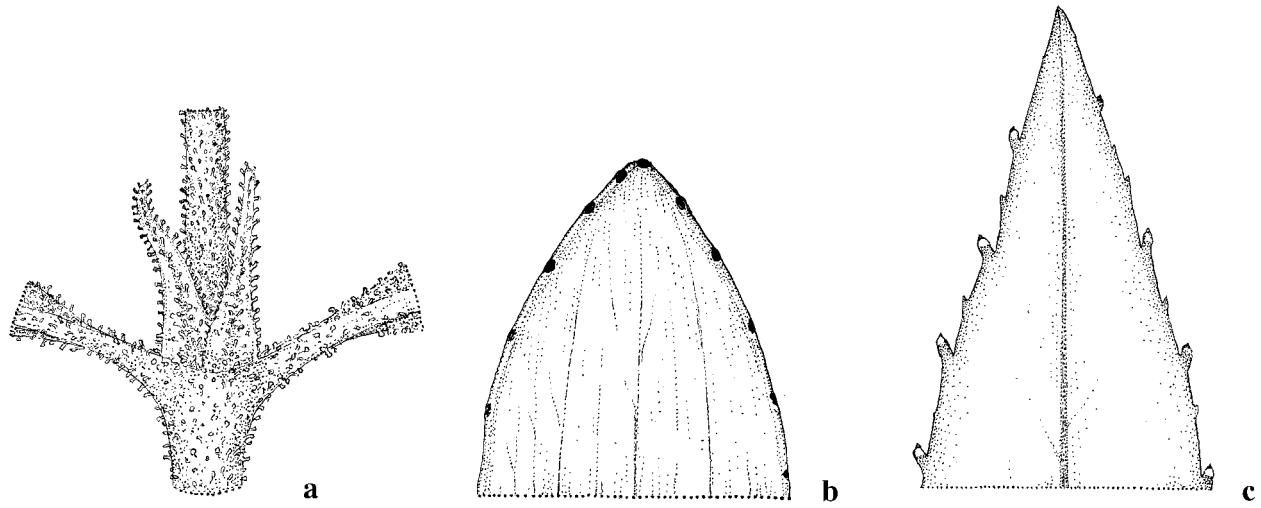

Fig. 6. Stipule and gland morphologies of Elatinaceae. (a) Paired interpetiolar stipules, *Bergia pedicellaris* F. Muell. from *Craven 6149* (MO), (b) marginal glands, *Elatine alsinastrum* Linn., from *Eggert* without collection number (MO), (c) marginal glands at apex of teeth, *Bergia glutinosa* Dinter & Schulze-Menz, from *Phipps 2906* (MO). Scale bar equals 5 mm.

cally and developmentally similar to the latex found in these malpighs. This should be examined in future anatomical comparisons of Elatinaceae and Malpighiaceae. Also, these two taxa share the presence of unicellular hairs, and although the conspicuous, medifixed T-shaped hairs found in Malpighiaceae (Fig. 1c) are not present in Elatinaceae, they are nevertheless unicellular (and also uniseriate in some Elatinaceae) in both taxa. Finally, both taxa often possess conspicuous, multicellular glands on their leaves. In Elatinaceae, these glands are usually borne along the leaf margin (Fig. 6b), and in some species of *Bergia*, they terminate small teeth along the margin (Fig. 6c). Similar extrafloral glands are commonly found in species of Malpighiaceae, and among those malpigh species with small teeth on their leaves, the teeth may similarly terminate in a gland or multicellular hair (W. R. Anderson, University of Michigan Herbarium, personal communication). This tooth is typically formed by receding tissue immediately adjacent to the marginal gland or cilium, which often gives the leaf margin a scalloped appearance.

Conclusions—This study helps resolve the placement of three problematic angiosperm families, for which the immediate sister taxa have been unknown. Peridiscaceae are not sister to Malpighiaceae as indicated in previous studies and should be excluded from the order Malpighiales; they are well supported as members of Saxifragales. Instead, Malpighiaceae form a strongly supported clade with Elatinaceae. These two sister taxa may have undergone a differential rate of diversification; there are ~1300 species of Malpighiaceae vs. only 35–50 species of Elatinaceae. Preliminary sister group comparisons of extant species diversity (Sims and McConway, 2003) between Elatinaceae (35 species) and Malpighiaceae (1250 species) indicate that diversification rates are significantly heterogeneous between these taxa ($\chi^2 = 3.679$; df = 1; $P = 0.05$).

One scenario to account for this asymmetry might be that there was an increase in diversification rates associated with the origin of malpighs, attributable to their unique floral morphology and coevolution with neotropical oil-bee pollinators. An alternative scenario is that there was a downshift in diversification rates associated with the aquatic habit exhibited by some Elatinaceae. Sister-group comparisons that are based on standing taxonomic diversity like those of Sims and McConway (2003) and others (e.g., Slowinski and Guyer, 1989; Guyer and Slowinski, 1993), however, are nondirectional (Sanderson and Wojciechowski, 1996, and references within) and do not allow us to discern whether there has been an increase in diversification associated with the origin of malpighs or if there was a decrease in Elatinaceae (or if both may have taken place). Until we can localize the shift in diversification on the phylogenetic tree, it is difficult to invoke a deterministic (''adaptive'') explanation to account for the observed differences of these two groups. Tests are available for identifying where shifts in diversification occur on phylogenies (e.g., Sanderson and Donoghue, 1994), but they require at least a three-taxon tree. Until the sister group of the Malpighiaceae/Elatinaceae clade is clarified, this will remain problematic. Examining these questions will require a better resolved phylogenetic assessment of Malpighiales as well as Elatinaceae to determine when members of the family made the shift to being completely aquatic and herbaceous. In the latter case, the aquatic and herbaceous members of Elatinaceae may be more recently evolved, whereas the terrestrial and woody members might be plesiomorphic within the family.

Note added in proof: As this paper was going to press, V. Savolainen, M. Cheek and M. Chase (K) discovered that *Soyauxia* (a previously unplaced eudicot in APG II, 2003) was placed with high bootstrap support as sister to *Peridiscus* using the three genes, *atpB*, *rbcL*, and 18S rDNA. This taxon had previously been allied to *Peridiscus* and *Whittonia* by Sandwith (1962) and Metcalfe (1962). In the future, Peridiscaceae should also include *Soyauxia*.

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